CENTRAL LUZON STATE UNIVERSITY

CYTOTOXICITY, ANTIBACTERIAL AND PHYTOCHEMICAL ANALYSIS OF SERPENTINA (*Andrographis paniculata* Nees) LEAVES EXTRACT

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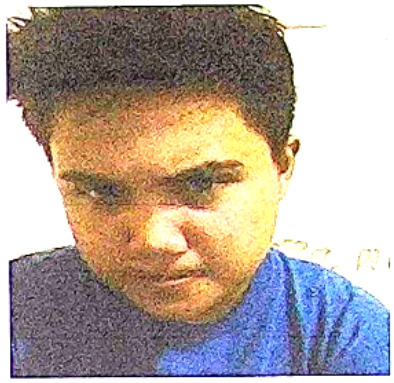

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ABSTRACT

JOSE, FRANCHESCA JENNINA V. Bachelor of Science in Biology, Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines, June 2017. **CYTOTOXICITY, ANTIBACTERIAL AND PHYTOCHEMICAL ANALYSIS OF SERPENTINA (*Andrographis paniculata* Nees) LEAVES EXTRACT**

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Andrographis paniculata Nees is a member of the family Acanthaceae that is commonly used by the local people, for the treatment of common cold, liver diseases, snake bite and some skin infection.

In this study, the cytotoxicity, antibacterial activity and phytochemical composition of the leaf extracts of *A. paniculata* were investigated. The cytotoxicity assessment was determined using brine shrimp assay and lethal concentration (LC50) of the extracts was analyzed using the probit analysis. The antibacterial activity was determined using agar disc diffusion method against *Staphylococcus aureus* and *Escherichia coli*. Chemical constituents of the plants were screened using the standard method of phytochemical analysis.

Results of our study revealed that cytotoxicity assay of both ethanol and hot water extract of *A. paniculata* considered as toxic having LC50 of 124.86µg/ml and 116.27µg/ml for both hot water and ethanol extracts respectively. In antibacterial activity,

both extracts did not inhibit the growth of both bacterial pathogen used. Results of phytochemical analysis showed that *A. paniculata* contained saponins, tannins, flavonoids, coumarins found in ethanol extract and tannins, terpenoids, coumarins in hot water extract.

LITERATURE CITED

AHMAD, I. MEHMOOD, Z. and F. MOHAMMAD. (1998). Screening of some Indian medicinal plants for their antimicrobial properties. Journal of Ethnopharmacology 62: 183-193.

AJAYI, I.A. AJIBADE, O. and R.A. ODERINDE. (2011). Preliminary phyto chemical analysis of some plant seeds. Research Journal of Chemical Sciences, 1: 58-62.

AKINPELU, D.A. ADEGBOYE, M.F. ADELOYE, O.A. and A.L. OKOH. (2008). Biocidal activity of partially purified fractions from methanolic extract of *Garcinia kola* (Heckel) seeds on bacterial isolates. Journal of Biology Resource 41: 277-287.

BATKHUU, J., HATTORI, K., TAKANO, F., FUSHIYA, S., OSHIMAN, K.I. and Y. FUJIMIYA. (2002). Suppression of NO production in activated macrophages in vitro and ex vivo by neoandrographolide isolated from *Andrographis paniculata*. Biological and Pharmaceutical Bulletin, 25:1169-1174.

BURGOS, R.A., LOYOLA, M., HIDALGO, M.A., LABRANCHE, T.P. and J.L. HANCKE. (2003). Effects of 14-deoxyandrographolide on calcium mediated rat uterine smooth muscle vontractility. Phytotherapy Research, 17:1011-1015.

BHAKUNI, D.S. DHAR, M.L. DHAWAN, B.N. and B.N. MEHROTRA. (1969). Screening of Indian plants for biological activity, part II. Indian Journal of Experimental Biology 7: 250-262.

CACERAS, D.D. HANCKE, J.L. BURGOS, R.L.A. and J.K. WICKMAN. (1997). Prevention of common cold with *Andrographis paniculata* dried extract. A pilot double blind trial. Phytomedicine. 4:101-104

CARSON, C. F. HAMMER, K.A. and T.V. RILEY. (1995). Broth microdilution method for determining the susceptibility of *Escherichia coli* and *Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia* (tea tree oil). Microbiology, 82: 181-185.

CAROCHO, M. and I.C.F.R. FERREIRA. (2013). A review on antioxidants, pro oxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. Food and Chemical Toxicology, 51:15-25.

CHANG, H.M. and P.P.H. BUT.(1987). Pharmacology and Applications of Chinese

Materia Medica. English translation by Shem Chang-Shing Yeung, Sih Cheng-Yao and Lai-Ling Wang (Chinese Medicinal Material Research Centre, The Chinese University of Hong Kong), Singapore: World Scientific Publishing Company Private Limited; 2:918-928.

CHAO, W.W. and B.F. LIN. (2010). Isolation and identification of bioactive compounds in *Andrographis paniculata* (*Chuanxinlian*). Chinese Medicine, 5:1-15.

CHAO, W.W., KUO, Y.H. and B.F. LIN. (2010). Anti-inflammatory Activity of New Compounds from *Andrographis paniculata* by NF-κB Trans-Activation inhibition. Journal Agricultural Food Chemistry, 58:2505-2512.

CHEN, L., ZHU, H., WANG, R., ZHOU, K., JING, Y. and F. QIU. (2008). Ent Labdane diterpenoid lactone stereoisomers from *Andrographis paniculata*. Journal of Natural Products,71:852-855.

COON, J. T. and E.T. ERNST. (2004). *Andrographis paniculata* in the treatment of upper respiratory tract infections: Assystemic review of safety and efficacy. Plantamadica, 70:293-298.

DENIAL, G. (2009). Medicinal uses of *Andrographis paniculata.* International Herb Association, 8-19.

DOUGHARI, J.H. (2006). Antimicrobial activity of *Tamarindus indica* Linn. Tropical Journal of Pharmaceutical Research, 5:597-603.

ELOFF, J.N. (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? Journal of Ethnopharmacology 60: 1-8.

EPAND, R.M., EPAND, R.F. and P.B. SAVAGE. (2007). Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds (Ceragenins). Biochimica et Biophysica Acta (BBA), 1768:2500-2509.

EVANS, W. C. (2002). Trease and Evans Pharmacognosy, W.B. Saunders, Edinburgh, London; 72.

FARNSWORTH, N. R. and D. D. SOEJARTO. (1991). Global importance of medicinal plants. In: Akerele, O., Heywood, V., and Synge, H. (Eds.), The Conservation of Medicinal Plants. Cambridge University Press, Cambridge: 25-51.

GABRIELIAN, E.S. SHUKARIAN, A.K. GOUKASOVA, G.I. CHANDANIAN, G.L. and A.G. PANOSSIAN. (2002). A double blind, placebo-controlled study

of *Andrographis paniculata* fixed combination Kan Jang in the treatment of acute upper respiratory tract infections including sinusitis. Phytomedicinal, 9:589-597.

GARBA, S. and S.O. OKENIYI. (2012). Antimicrobial activities of total alkaloids extracted from some Nigerian medicinal plants. Journal of Microbiology and Antimicrobials, 4:60-63.

GAO, Y., VAN BELKUM, M. J. and M. E. STILES. (1999). "The outer membrane of Gram negative bacteria inhibits antibacterial activity of brochocin-C"; Applied and Environmental Microbiology, 65, 4329–4333.

GEETHANGILI, M., RAO, Y.K., FANG, S.H. and Y.M. TZENG. (2008). Cytotoxic constituents from *Andrographis paniculata* induce cell cycle arrest in Jurkat cells. Phytotherapy Research, 22:1336-1341.

GUERIN-FAUBLEE, V., MULLER, M.L.D., VIGNEULLE, M. And J.P. FLANDROIS. (1996). Application of a modified disc diffusion technique to antimicrobial susceptibility testing of *Vibrio anguillarum* and *Aeromonas salmonicida* clinical isolates. *Veterinary Microbiology* **51**: 137-149.

GUPTA, R.K., KESARI, A.N., MURTHY, P.S., CHANDRA, R., TANDON, V. and G. WATAL. (2005). Hypoglycemic and antidiabetic effect of ethanolic extract of leaves of *Annona squamosa*L. in experimental animals. Journal of Ethnopharmacology., Lausanne, 99, 75-81.

HARRIS, L.G., FOSTER, S.J. and R.G. RICHARDS. (2002). An introduction to *Staphylococcus aureus*, and techniques for identifying and quantifying *S. aureus* adhesins in relation to adhesion to biomaterials: review. European Cells and Materials, 4:39-60.

HEROUART, D., SANGWAN, R.S., FLINIAUX, M.A. and B.S. SANGWAN NORREEL. (1988). Variations in the Leaf Alkaloid Content of Androgenic Diploid Plants of Datura innoxia. Planta Medica, 54: 14-17.

HUANG, Y.H., CHANG, H.C. and T.C. CHANG. (2001). Development of a latex agglutination test for rapid identification of *Escherichia coli*. European Journal of Clinical Microbiology and Infectious Diseases, 20: 97-103.

JADA, S.R., SUSENO, G.S., MATTHEWS, C., HAMZAH, A.S., LAJIS, N.H., SAAD, M.S., STEVENS, M.F.G. and J. STANSLAS. (2007). Semisynthesis and in vitro anticancer activities of andrographolide analogues. Phytochemistry, 68:904-912.

JAYASHREE, D. (2013). Phytochemicals analysis and TLC fingerprinting of methanolic extracts of three medicinal plants. International Research Journal of Pharmacy, 4:123-126.

JI, L.L., WANG, Z., DONG, F., ZHANG, W.B. and Z.T. WANG. (2005). Andrograpanin, a compound isolated from anti-inflammatory traditional Chinese medicine *Andrographis paniculata*, enhances chemokine SDF-1α-induced leukocytes chemotaxis. Journal of Cellular Biochemistry, 95:970-978.

KALEMBA, D. and A. KUNICKA. (2003). "Antibacterial and antifungal properties of essential oils"; Current Medicinal Chemistry, 10: 813–829.

KAPIL, A., KOUL, I.B., BANERJEE, S.K. and B.D. GUPTA. (1993). Antihepatotoxic effects of major diterpenoid constituents of *Andrographis paniculata*. Biochemistry Pharmacological, 46:182-185.

KAPPERS, I.F., AHARONI, A., VAN HERPEN, T.W., LUCKERHOFF, L.L. and M. DICKE. (2005). Genetic engineering of terpenoid metabolism attracts bodyguards to Arabidopsis. Science, 309: 2070-2072.

KINGHORN, A.D., PAN, L., FLETCHER, J. N. and H. CHAI. (2011). The relevance of higher plants in lead compound discovery programs. Journal of Natural Products. 74: 1539-1555.

KUMAR, A., DORA, J., SINGH, A. and R. TRIPATHI. (2012). A review on king of bitter (Kalmegh). International journal of research in pharmacy and chemistry, 2:116- 124.

KRISHNAIAH, D., SARBATLY, R. and A. BONO. (2007). Phytochemical antioxidants for health and medicine: A move towards nature. Biotechnology and Moecularl Biology Reviews, 1: 97-104.

LENZEN, S., TRIPARTHI, H.C., and S.K. TANDON. (1998). Antidiabetic Activity of Andrographolide Indian Journal of Pharmacology,18,58-61.

LEVITA, J., NAWAWI, A., MUTALIB, A. and S. IBRAHIM. (2010). Andrographolide: A review of its anti-inflammatory activity via inhibition of NF-kappaB activation from computational chemistry aspects. International Journal of Pharmacology, 6:569-576.

LIU, J., WANG, Z.T., JI, L.L. and B.X. GE. (2007). Inhibitory effects of neo andrographolide on nitric oxide and prostaglandin E2 production in LPS-stimulated murine macrophage. Molecular and Cellular Biochemistry, 298:49-57.

LIU, J., WANG, Z.T. and B.X. GE. (2008). Andrograpanin, isolated from *Andrographis paniculata*, exhibits anti-inflammatory property in lipopolysaccharide induced macrophage cells through down regulating the p38 MAPKs signaling pathways. International Immunopharmacology, 8:951-958.

MATSUDA, T., KUROYANAGI, M., SUGIYAMA, S., UMEHARA, K., UENO, A. and K. NISHI. (1994). Cell differentiation inducing diterpenes from *Andrographis paniculata* Nees. Chemical and Pharmaceutical Bulletin (Tokyo), 42:1216-1225.

MAHATO, S.B. and S. SEN. (1997). Advances in triterpenoid research, 1990-1994.Phytochemistry, 44: 1185-1236.

MARLES, R.F. and N.R. FANSWORTH. (1995). Antidiabetic plants and their active constituents. Phytomedicine, Stuttgart, 2, 137-189.

MEYER, B.N., FERRIGNI, N.R., PUTNAM, J.E., JACOBSEN, L.B., NICHOLS, D.E. and J.L. MCLAUGHLIN. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. Planta Medica, 45:31-33.

MOHAMED SHAM SHIHABUDEEN, H., HANSI PRISCILLA, D. and K. THIRUMURUGAN. (2010). Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. International Journal of Pharmaceutical Sciences and Research, 1:430-434.

MCLAUGHLIN, J.L., CHANG, C.J. and D.L. SMITH. (1991). in A.-U. Rahman (Ed.), Studies in Natural Products Chemistry, "bentch-top" Bioassays for the Discovery of Bioactive Natural Products: an Update, Structure and Chemistry (Part-B), vol. 9, Elsevier Science Publishers, The Netherlands, 383.

MCLAUGHLIN, J.L., CHANG, C.J. and D.L. SMITH. (1991). in ÒStudies in Natural Products ChemistryÓ, Attaur-Rahman (Ed.), Elsevier Science Publishers B.V., Amsterdam, 9, 383-409.

MCLAUGHLIN, J.L., CHANG, C.J. and D.L. SMITH. (1991). Bench top" bioassay for the discovery of bioactive natural products: an update. In: Studies in Natural Products Chemistry (Ed.), Rahman A.U., Elsevier, 383-409.

MCLAUGHLIN, J.L., CHANG, C.J. and D.L. SMITH. (1993). Simple bench-top bioassays (brine shrimp and potato discs) for the discovery of plant antitumour compounds. In: Human Medicinal Agents from Plants. Kinghorn, A. D. and Balandrin, M. F. (Eds.), ACS Symposium 534, American Chemical Society, Washington, D. C.:112-137.

NEGRI, G. (2005). Diabetes melito: plantas e princípiosativosnaturaishipoglicemiantes. It Revista Brasileira de Ciencias Farmaceuticas São Paulo, 41, 121-142.

OKEKE, M.I., IROEGBU, C.U., EZE, E.N., OKOLI, A.S. and C.O. ESIMONE. (2001). Evaluation of the Extracts of the Roots of Landolphia Owerrience for Anti-bacterial activity, Journal of Ethanopharmacology, 78:119-127.

OKHUAROBO, A., FALODUN, J.E., ERHARUYI, O., IMIEJE, V., FALODUN, A. and P. LANGER. (2014). Harnessing the medicinal properties of *Andrographis paniculata* for diseases and beyond: a review of its phytochemistry and pharmacology. Asian Pacific Journal of Tropical Disease, 4:213-222.

OTANG, W.M., GRIERSON, D.S. and R.N. NDIP. (2013). Assessment of potential toxicity of three South African medicinal plants using the brine shrimp (*Artemia salina*) assay. African Journal of Pharmacy Pharmacology;7:1272-9.

POOLSUP, C., SUTHISISANG, C., PRATHANTURARUG, S., ASAWAMEKUN, A. and U. CHANCHAREON. (2004). *Andrographis paniculata* in the symptomatic treatment of uncomplicated upper respiratory tract infection: systematic review of randomized controlled trials. Journal of Clinical Pharmacy and Therapeutics, 29:37-45.

RADHA, R., SERMAKKANI, M. and V. THANGAPANDIAN. (2011). Evaluation of phytochemical and antimicrobial activity of *Andrographis paniculata* Nees (Acanthaceae) aerial parts. International Journal of Pharmacy and Life Sciences, 2:562-567.

RAETZ, C. R. H. and C. WHITFIELD. (2002). Lipopolysaccharide endotoxins. Annual Review Biochemistry, 71: 635-700.

RAHMAN, N.N.N., FURUTA. A., KOJIMA, T., TABANE, S. and M. ALI-MOHD. (1999). Anti-malarial activity of extracts of Malaysian medicinal plants Journal of Ethnopharmacology, 64: 249-254.

RAMASAMY, S, and M.A. CHARLES. (2009). Antibacterial effect of volatile components of selected medicinal plants against human pathogens. Asian Journal of Microbial Biotechnology Environmental, 6:209–10.

RISS, T.L., and R.A. MORAVEC. (2004). "Use of multiple assay end points to investigate the effects of incubation time, dose of toxin, and plating density in cell-based cytotoxicity assays". Assay Drug Development and Technology, 2:51-62.

SABAI, K.K., THIN, N.N., SHWE, K. and T.M. HTWE. (2001). Evaluation of the

bioactivities of some Myanmar medicinal plants using brine shrimp (*Artemia salina*) toxicity test. Journal of Myanmar Academic Technology;1:105-12..

SAM, T. W. (1993). Toxicity testing using the brine shrimp: *Artemiasalina*. In: Colegate, S. M. and Molyneux, R. J. (Eds.), Bioactive Natural Products Detection, Isolation, and Structural Determination. CRC Press, Boca Raton, FL: 442-456.

SAMY, R.P. THWIN, M.M. GOPALAKRISHNAKONE, P. and S. IGNACIMUTHU. (2008). Ethnobotanical survey of folk plants for the treatment of snake bites in southern part of Tamilnadu. India. Journal of Ethnopharmacology, 115:302-312.

SAREER, O. AHAD, H. and S. UMAR. (2012). Prophylactic and lenitive effects of *Andrographis paniculata* against common human ailments: An exhaustive and comprehensive reappraisal. Journal of Pharmaceutical Research and Opinion, 2: 138-162.

SIBANDA, T. and A.I. OKOH. (2008). In vitro antibacterial regimes of crude aqueous and acetone extract of *Garcinia kola* seeds. Journal of Biology Science, 8: 149-154.

SIVANANTHAN, M. and M. ELAMARAN. (2013). Medicinal and pharmacological properties of *Andrographis paniculata*. International Journal of Biomolecules and Biomedicine, 3:1-12.

SULE, A., AHMED, Q.U., SAMAH, O.A. and M.N. OMAR. (2011). Bacteriostatic and bacterial activities of *Andrographis paniculata* extracts on skin disease causing pathogenic bacteria. Journal of Medical Plant Research, 5:7-14.

SHAIK, D., MALIKA, F.A., RAFI, S.M. and B. NAQUI. (1994). Studies of antibacterial activity of ethanolic extract from *Nericum indicum* and *Hibiscus rosa sinensis*. Journal of Islamic Academy Sciences, 7:167–8.

SHELTON, R.M. (1991). *Aloe vera*: its chemical and therapeutic properties International Journal of Dermatology, 30:679-683.

SHEN, Y.C., CHEN, C.F. and W.F. CHION. (2000). Suppression of rat neutrophil reactive oxygen species production and adhesion by the diterpenoid lactone andrographolide. Planta Medica 66: 314-317

TAPSELL, L.C., HEMPHILL, I., COBIAC, L., PATCH, C.S., SULLIVAN, D.R., FENECH, M., ROODENRYS, S., KEOGH, J.B., CLIFTON, P.M., WILLIAMS, P.G., FAZIO, V.A. and K.E. INGE. (2006). Health benefits of

herbs and spices: the past, the present, the future. Medical Journal of Australia 21: S4-24.

THIYAGARAJAN, P., DEEPAK, H.B. and A. AGARWAL. (2011). In vitro modulation of LPS/calcimycin induced inflammatory and allergic mediators by pure compounds of *Andrographis paniculata* (King of bitters) extract. International Immuno pharmacology. 11:79-84.

WINSTANLEY, T., EDWARDS, C., LIMB, D., MEGSON, K. and R.J. SPENCER. (1994). Evaluation of a surfactant, Dispersol LN, as an anti-sawrming agent in agar dilution susceptibility testing Journal of Antimicrobial Chemotherapy 33, 352-6.

YADAV, R.N.S. and M. AGARWALA. (2011). Phytochemical analysis of some medicinal plants. Journal of Phytology, 3:10-14.

YOOPAN, N., THISODA, P., RANGKADILOK, N., SAHASITIWAT, S., PHOLPHANA, N. and S. RUCHIRAWAT. (2007). Cardiovascular effects of 14-deoxy-11,12- didehydroandrographolide and *Andrographis paniculata* extracts. Planta Medica, 73:503-511.

ZHANG, C.Y. and B.K. TAN. (1997). Mechanism of cardiovascular acrivity of *Andrographis paniculata* in the anaesthetized rat. Journal of Ethnopharmacology, 56:97-101.

ZHANG, C.Y. and B.K. TAN. (1998). Vasorelaxation of rat thoracic aorta caused by 14-deoxyandrographolide. Clinical and Experimental Pharmacology and Physiology, 25:424-429.

ZHANG, C.Y. and B.K. TAN. (1999). Effects of 14-deoxyandrographolide and 14 deoxy- 11,12-didehydroandrographolide on nitric oxide production in cultured human endothelial cells. Phytotherapy Research, 13:157-159.